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SHORT COMMUNICATION

Presence of bla_{PER-1} and bla_{VEB-1} beta-lactamase genes among isolates of *Pseudomonas aeruginosa* from South West of Iran

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Abstract *Pseudomonas aeruginosa* isolates have acquired resistance to antibiotics such as novel beta-lactams. The aim of this study was to investigate the bla_{PER-1}, bla_{VEB-1}, and bla_{PSE-1} genes among isolates of *P. aeruginosa* among intensive care unit (ICU) patients. Sixty-five isolates were collected. The antibiotic susceptibility testing and combined disk tests were performed to detect the isolates producing extended spectrum beta-lactamases (ESBLs) among ceftazidime-resistant isolates. Polymerase chain reaction (PCR) amplification of bla_{PER-1}, bla_{VEB-1}, and bla_{PSE-1} genes was conducted. Ten (15.3%) isolates were ESBL-positive, of which 40% ($n = 4$) belonged to males and 60% ($n = 6$) were collected from females. Moreover, two and one isolates harbored bla_{PER-1} and bla_{VEB-1} genes, respectively.
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1. Introduction

Among several acquired beta-lactamase enzymes, the bla_{PER-1} and bla_{VEB-1}, although produced less frequently, have clinical importance because of conferring resistance to oxyimino beta-lactams

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[1]. Resistance to carbapenems is also of high concern, because of the spectrum of activity against Gram-negative and Gram-positive isolates. In *Pseudomonas aeruginosa*, similar to *Klebsiella pneumoniae* and *Acinetobacter baumannii*, a combination of several mechanisms contributes to the high level of resistance to carbapenems [2]. The aim of this study was to detect the genes encoding Class A extended spectrum beta-lactamases (ESBLs) of bla_{PER-1}, bla_{VEB-1}, and bla_{PSE-1} among clinical isolates of *P. aeruginosa* in intensive care unit (ICU) patients.

2. Material and methods

2.1. Antibiotic susceptibility and ESBL testing

A total of 65 clinical isolates of *P. aeruginosa* were collected from ICU patients and different infection sites in hospitals in Tehran (n = 11), Shiraz (n = 12), Kermanshah (n = 10), Ilam (n = 8), Kerman (n = 7), and Ahvaz (n = 17) in Iran between 2009 and 2011.

The antibiotic susceptibility testing was conducted according to Clinical and Laboratory Standards Institute 2012 guidelines. The antibiotics disks were included as previously described. Briefly, a swab of 0.5 McFarland was cultured on Müeller—Hinton agar and the disks were ordered following the Kirby Bauer method [3]. The combined disk test was performed with ceftazidime and cefotaxime with or without clavulanic acid placed on Müeller—Hinton agar media (containing cloxacillin).

2.2. Polymerase chain reaction amplification of bla_{PER-1}, bla_{VEB-1}, and bla_{PSE-1} genes

The polymerase chain reaction (PCR) was performed for the amplification of bla_{PER-1}, bla_{VEB-1}, and bla_{PSE-1} genes with specific primers.

The reaction mixture (PCR master mix) included: 10× PCR buffer = 2.5 μL, $MgCl_2$ (50 mM) = 1.5 μL, di-nucleotide triphosphate (dNTP) (10 Mm) = 0.75 μL, forward and reverse primers (each with 100 μM) = 2.5 μL, Taq DNA polymerase (5 U/μL) = 0.2 μL, template (DNA) = 1 μL, and nuclease-free H_2O = 14.05 μL (Sigma, Tehran province, Tehran, Iran). The PCR amplification conditions of the bla_{PER-1}, bla_{VEB-1}, and bla_{PSE-1} genes are added in Table 1. The Tris-Acetate-EDTA (TAE) buffer (EDTA 0.5 M, glacial acetic acid, and Tris) was used for the electrophoresis of products. The Student t test was used for analysis, and $p < 0.05$ was considered as significant.

Table 1 The prevalence of ESBL-positive isolates and the presence of genes among several investigated hospitals.

Hospital	ESBL-positive	bla_{PER-1}	bla_{VEB-1}
Tehran (n = 11)	4	0	0
Shiraz (n = 12)	3	0	0
Kermanshah (n = 10)	0	0	0
Ilam (n = 8)	0	0	0
Kerman (n = 7)	0	0	0
Ahvaz (n = 17)	3	2	1
Total	10	2	1

ESBL=.

3. Results

Of 10 ESBL-positive ICU isolates, 20% (n = 2) harbored the bla_{PER-1} gene (925 bp), which occurred in Ahwaz hospital in the South West of Iran. Moreover, one isolate amplified the bla_{VEB-1} (with 634 bp) in Ahwaz Hospital. The presence of two bla_{PER-1} genes was demonstrated in two isolates with panantibiotic resistance in urine samples.

4. Discussion

About half of the isolates in this study were resistant to the third generation cephalosporins, but we detected only 2 bla_{PER-1}- and one bla_{VEB-1}-positive isolates, indicating that the presence of other enzymes, such as Amp-C, ESBLs, and metallo-beta-lactamases or mechanisms including efflux pumps for cephalosporin resistance may be cooperated in this phenomenon. Interestingly, although 44 isolates were ceftazidime-resistant *P. aeruginosa*, 10 isolates were ESBL producers. Several previous studies obtained the same results. The presence of other mechanisms of resistance or other enzymes is possible. This is the first report of these beta-lactamases in the South West of Iran. The two bla_{PER-1}- and one bla_{VEB-1}-positive isolates were collected from urine samples in one hospital ICU setting of Ahvaz city and also for two bla_{PER-1}-positive isolates, the antibiotic susceptibility profile was the same, suggesting the occurrence of related isolates. Of the 10 ESBL-positive isolates, 40% (n = 4) belonged to males and 60% (n = 6) were collected from female patients. Furthermore, none of the ESBL producers amplified the bla_{PSE-1} gene. The distribution of ESBL producers and genes among several hospitals of the country (Tehran, Shiraz, Kermanshah, Ilam, Kerman, and Ahvaz) is demonstrated in Table 1.

Conflicts of interest

The authors have no conflicts of interest to declare.

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